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☐ Additional inventors are being named on the \_\_\_\_\_ separately numbered sheets attached hereto
**TITLE OF THE INVENTION (500 characters max)****LUPUS ANTIBODIES FOR PASSIVE IMMUNOTHERAPY OF HIV/AIDS**

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Application Data Sheet. See 37 CFR 1.76

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**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

Provisional Application

## **Lupus Antibodies for Passive Immunotherapy of HIV/AIDS**

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### **Introduction**

This invention describes the preparation of broadly reactive antibodies that bind to a conserved determinant of gp120 for the purpose of immunotherapy of HIV and immunoprophylaxis against HIV.

Recombinant antibody fragments derived from lupus patients who are not infected with HIV neutralize diverse strains of HIV belonging to clades B, C and D. These antibody fragments recognize a comparatively conserved determinant of HIV that is a constituent of the binding site of HIV coat protein gp120 for host cell CD4 receptors. The antibody fragments which neutralize HIV include single chain Fv constructs (VL and VH domains linked via a peptide linker) and light chain subunits. Intact antibody molecules that retain the properties of the Fv constructs can be readily isolated by methods well known in the art, such as (e.g., US patents 6,407,213 and 5,807,715). Improvements in the activity can be achieved by mutagenesis of the antibody genes and identification of the mutant proteins with the highest HIV binding and neutralizing activity (e.g, US patents 5,811,238 and 6,406,863).

The antibody fragments of this invention were isolated by phage display and selection for binding to the desired target antigen. This provisional application includes the attached manuscript entitled *HIV-1 Neutralizing Antibody Fragments to a Conserved Envelope Determinant from Lupus Libraries*, Karle *et al.*) and describes the phage display technique. Other, more routine methods of antibody isolation also allow isolation of the desired antibodies.

The lupus antibodies fragments are monovalent, and they display HIV neutralizing potency comparable to the best monovalent anti-HIV antibodies described in the literature. Recloning of

the monovalent fragments as bi- and multivalent fragments allows increased neutralizing potency due to avidity effects, as described for other antibodies in the literature. The human origin of these antibodies is a favorable factor, as this obviates anti-antibody responses.

The present antibodies and fragments are useful for passive immunotherapy in HIV-1 infected patients. Immunoprophylactic applications such as inclusion of the antibodies in a contraceptive cream are possible. Literature describing the use of antibodies in passive immunotherapy of HIV/AIDS are U.S. Patents 5,445,960; 5,695,927; and 5,783,670, incorporated herein by reference in their entirety. Additional patents describing standard methods for producing and using antibodies (e.g., b12 and X5) (Burton, Parren and Barbas from Scripps Institute, San Diego) and certain antibodies derived from hybridoma technology (Susan Zolla-Pazner group), are also incorporated herein by reference in their entirety.

This invention permits potent neutralization of HIV-1 across several clades. The neutralizing potencies are comparable or better than published values for other monovalent anti-HIV antibodies. In addition to the lupus clones described in the enclosed manuscript, additional Fv clones have recently been identified that display HIV neutralizing activity at concentrations as low as 0.025 microgram/ml (clones Fv JL 363 and Fv JL 379). These clones were isolated and analyzed for gp120 binding and HIV neutralization essentially as described in detail for Fv JL413 and JL427 here.

**Lupus and HIV.** Expression of retroviral genes which have migrated to the human genome has been implicated in the etiology of autoimmune disease (98). Antibodies to gp120 are found in lupus patients and mouse models of lupus (99-101). Epidemiological studies have noted the rare coexistence of lupus and AIDS (102-104). These considerations impelled us to isolate recombinant Ab fragments capable of binding gp120 from lupus phage display libraries for study of HIV neutralizing characteristics. Using selection and immunochemical procedures designed to isolate antibodies that recognize the gp120 determinant composed of residues 421-436, we succeeded in isolating several Ab fragments that neutralize each of the primary isolates of HIV-1 analyzed in PBMC cultures, belonging to clades B, C and D. Neutralization potency of the lupus

Ab fragments compare favorably with Abs proposed in the literature as promising immunotherapy candidates (5,6).

The Ab fragments are monovalent (Fv/L chains). Recloning these molecules as bivalent IgG improves neutralization potency even further due to avidity effects. Replacement mutations were clustered in the CDRs (compared to the FRs), which is a hallmark of affinity matured V genes encoding high affinity Ag binding antibodies. Thus, the lupus Ab repertoire is a source of neutralizing Abs to HIV-1.

Previously, we observed that polyclonal IgG preparations from autoimmune MRL/lpr mice (unimmunized) cleaved 125Igp120 (SF2 strain). Similarly, L chains isolated from polyclonal human lupus patients cleaved gp120, but a similar activity was not found in HIV-1 positive patients known to express gp120 binding Abs. One of 29 monoclonal L chain isolated from multiple myeloma patients was observed to cleave gp120 (31). Whether gp120 itself or a gp120-like retroviral Ag is the immunogen responsible for stimulating the formation of these Abs remains conjectural. The alternative, is that a self-Ag with homology to gp120 is the inciting immunogen. Homologies between certain self-Ags (HLA class I heavy chains, VIP and neuroligin) are known (105-107), but these homologies do not fall within gp120 residues 431-436.

Regardless of the inciting immunogen, the lupus anti-gp120 Abs ought to be valuable in HIV therapy. Therefore, we subjected the Fv and L chain phage libraries to covalent selection on the gp120(421-436)-CRA. Abs capable of cleaving monomer gp120 have also been isolated successfully by this strategy, some of which are potent inhibitors of HIV infection. Further studies are needed to determine the role of catalysis in the mechanism of neutralization. For instance, it is necessary to assess cleavage of native trimeric gp120 (as opposed to monomer gp120) to obtain useful correlations with HIV neutralization.

**Lupus anti-HIV Ab fragments.** Abs that bind the comparatively conserved epitope of gp120 composed of residues 421-436 are found in lupus (99,110). Amino acids within this determinant contribute important contacts in binding of HIV-1 by host cell CD4 receptors. Lupus Abs appear to recognize native trimeric gp120, indicated by observations of immunofluorescent staining of HIV infected cells with IgG purified from aged mice with lupus (MRL/lpr strain, these mice have a deficient Fas receptor gene; the resultant defect in lymphocyte apoptosis produces lupus-like autoimmune disease). The anti-gp120(421-436) binding Abs appear in the serum of the MRL/lpr mice as a function of age, concomitant with the onset of symptoms of autoimmune disease.

Human and murine lupus are associated with what appears to be an overall tendency towards synthesis of Abs with catalytic activity. Study of polyclonal lupus IgG indicated the presence of gp120-cleaving Abs. IgG samples purified by affinity chromatography from 17 HIV-1 positive patients showed little or no cleavage of 125I-gp120 by HIV IgG samples, but the 3 of 10 lupus IgG samples cleaved the protein, evident as depletion of the intact gp120 band and formation of smaller radioactive bands in SDS-gels. In separate experiments, L chains were purified from one of the gp120 cleaving lupus IgG samples (code 530) and from the serum IgG of lupus MRL/lpr mice (by reduction/alkylation of the IgG and FPLC gel filtration using previously described protocols, (89)). The 125I-gp120 cleaving activity was evident in the fractions corresponding to the L chain peak from both the lupus patient and the MRL/lpr mice (25 kD) (110).

The identity of the L chains recovered from the FPLC column was confirmed by SDS-PAGE and immunoblotting as described previously. In the presence of a serine protease inhibitor (0.3 mM DFP), gp120 cleavage by IgG from a lupus patient was essentially completely inhibited. In comparison, inhibitors of metalloproteases, cysteine proteases and acid proteases (EDTA, iodoacetamide, Pepstatin A) were without effect. Recombinant Fv and L chains clones capable of binding gp120 noncovalently and catalyzing its cleavage were isolated from human lupus libraries by various strategies: (i) Fv phage noncovalent binding to gp120 conjugated to Affigel with pH2.7 elution (40); (ii) Fv phage covalent binding to whole biotinylated gp120-CRA VIb followed by separation of the bound phage on an anti-gp120 column; (iii) L chain

phage noncovalent binding to immobilized gp120(421-436); and (iv) L chain phage covalent binding to immobilized gp120(421-436)-CRA Vc followed by recovery of bound phages by reduction of the S-S bond placed located in this CRA (45). Soluble Fv fragments were obtained by expression of the selected phagemid DNA (periplasm of HB2151 cells; this bacterial host reads a codon located between the Ab insert and the p3 protein as a stop codon, allowing soluble expression of the recombinant proteins). These were analyzed for: (i) binding to full-length gp120 (monomeric) and synthetic gp120(421-436) conjugated to albumin; (ii) cleavage of monomer gp120, (iii) neutralization of HIV-1. Noteworthy results are: (a) Following phage noncovalent selections, increased binding of gp120 binding and gp120(421-436) binding was evident in the Fv and L chain populations, indicating the success of selection procedures. With the exception of one clone, binding of gp120 and gp120(421-436) were correlated. Dose-dependent antigen binding by the purified Ab fragments was observed. Binding of one of the clones to irrelevant proteins (calmodulin, thyroglobulin, albumin) was analyzed. No significant recognition of these proteins was observed. Two important conclusions may be drawn. First, determinant 421-436 in the synthetic peptide and full-length gp120 must share substantial structural similarity. Second, most gp120 binding Abs in lupus are directed at recognition of the gp120(421-436) epitope. Immunogenetic analysis of lupus Abs has previously shown that some of these do not contain sufficiently diversified V domain sequences to anticipate Ag-specific binding activities (such Abs usually display polyreactive binding patterns). This was not the case for the gp120-binding Abs. Sequence analysis of the two Fv clones and one L chain indicated the presence of extensive replacement mutations compared to their germline V gene counterparts (Table 2). The mutations tended to cluster in the complementarity determining regions (versus framework region of the V domains), and the ratio of replacement to silent mutation was generally greater for the CDRs than the FRs. This is a sign that the Ab V domains have been subjected to adaptive sequence diversification typical of B cell clonal selection. The purified Fv and L chain clones isolated by noncovalent binding techniques neutralized primary isolates of HIV-1 with impressive potency. This was evident from measurement of infection of peripheral blood mononuclear cells (PBMC) by three primary isolates of HIV-1 belonging to clades B, C and D. Inhibition of infection was not due to a cytotoxic effect, as no loss of cell viability was observed following incubation with Ab fragments in the absence of HIV (determined by staining

cells with acridine orange/ethidium bromide; Sigma; viability ~80-85% in cells treated with diluent and the Ab preparations). No neutralization of HIV-1 was observed in the presence of equivalently purified irrelevant control Fv and L chain clones or purified extracts of bacteria harboring vector devoid of Ab inserts were also devoid of inhibitory activity. IC50 for Fv clone 683 is 6 ng/ml (strain ZA009). This compares favorably with the neutralization potency of candidate immunotherapeutic Abs described in the literature (e.g., Fab fragments of Ab b12; other bivalent IgG molecules isolated from HIV+ subjects or derived from in vitro molecular evolution strategies. (b) Two types of covalent phage selections indicate the feasibility of HIV-1 targeting by specific gp120-cleaving Abs from the lupus repertoire. First, gp120 peptide-CRA Vc was applied for selecting phage L chains. Phage-CRA complexes formed by mixing these reagents were trapped on a streptavidin column and the phage subpopulation complexed irreversibly to the CRA (i.e., phage subpopulation not removed by pH2.7 and pH11 buffer washes) were recovered by cleaving the S-S bond located between the biotin and the phosphonate moiety. Initial catalysis assays utilized the substrate gp120(421-432)-methylcoumarinamide (MCA; the targeted bond is K432-MCA, located at the position of the phosphonate moiety in CRA Vc). Measurement of aminomethylcoumarin release (by fluorimetry as in our previous studies), suggested a catalytic activity in the recovered clones.



P.I. Paul, S.

51

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55

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**Claims**

1. Antibodies or antibody fragments that neutralize HIV-1 derived from patients with autoimmune disease.
2. Antibodies or antibody fragments that neutralize HIV-1, derived from patients with lupus.
3. The antibodies of claim 1 or 2, which are monoclonal antibody fragments obtained by cloning cDNA for the antibody variable domains of the light chain (VL) and heavy chain (VH) from mRNA expressed by lymphoid cells.
4. The monoclonal antibody fragments of claim 3 obtained as single chain Fv constructs comprising the VL and VH domains linked by a flexible peptide linker.
5. The antibody fragments of claim 4 in which the order of arrangement of the components from N terminus to C terminus is VL-linker-VH
6. The antibody fragments of claim 4 in which the order of arrangement of the components from N terminus to C terminus is VH-linker-VL.
7. The antibody fragments of claim 4 in which the amino acid constitution of the linker is varied by mutagenesis to optimize the VL-VH interface supporting the greatest HIV neutralizing activity.
8. The antibody fragments of claim 4 in which the length of the linker is varied by mutagenesis to optimize the VL-VH interface supporting the greatest HIV neutralizing activity.
9. Monoclonal antibody light chain subunits of claim 1, obtained by cloning the cDNA for VL and light chain constant regions (CL).
10. Monoclonal Fv constructs of claim 4 reactive with HIV, obtained by expression of the Fv constructs on the surface of phage particles and identifying the subpopulation of HIV-reactive Fv particles by binding to intact HIV, trimeric gp120, monomer full-

length gp120 and synthetic peptide fragments of gp120 .

11. The monoclonal Fv constructs of claim 10 which neutralizes at least three strains belonging to different HIV clades.
12. The monoclonal Fv constructs of claim 10, which neutralize strains belonging to HIV-1 clades B, C and D.
13. Monoclonal full-length immunoglobulins obtained by recloning Fv fragments of claim 11 as IgG, IgA or IgM constructs.
14. Monoclonal light chains of claim 9 reactive with HIV, obtained by expression of the light chain on the surface of phage particles and identifying the subpopulation of HIV-reactive light chain particles by binding to intact HIV, trimeric gp120, monomer full-length gp120 or synthetic peptide fragments of gp120 .
15. Monoclonal light chains of claim 14, which neutralize at least three strains belonging to different HIV clades.
16. Monoclonal light chains of claim 14, which neutralize strains belonging to HIV-1 clades B, C and D.
17. Monoclonal Fv constructs containing the VL domains from light chains of claim 15 and 16 paired with the VH domain of other anti-gp120 antibodies.
18. Monoclonal full-length immunoglobulins obtained by recloning Fv fragments of claim 17 as IgG, IgA and IgM constructs.
19. Monoclonal full-length immunoglobulins with binding specificity for HIV obtained by preparing a plurality of hybridomas of lymphoid cells from lupus patients and myeloma cells and screening said hybridomas for secretion of antibodies for HIV binding capability and HIV neutralizing activity.
20. Monoclonal full-length immunoglobulins of claims 13, 18 and 19, which neutralized

at least three strains belonging to different HIV clades and subtypes.

VERSION 03-18-03

## **HIV-1 Neutralizing Antibody Fragments to a Conserved Envelope Determinant from Lupus Libraries**

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*Running title:* Lupus anti-HIV antibodies

**ABSTRACT**

Cross-clade neutralizing antibodies (Abs) to conserved HIV-1 components are highly sought because of their potential use in immunotherapy of AIDS. HIV-1 infection occurs only rarely in lupus patients. We hypothesized that intrinsic immunological responses in lupus patients protect against HIV infection. Phage displayed single chain Fv and light chain libraries from lupus patients were selected and screened by procedures allowing isolation of clones capable of binding coat protein gp120 residues 421-436, a comparatively conserved component of the CD4 binding site. Binding of synthetic gp120(421-436) and full-length gp120 by the Ab fragments was correlated, indicating the peptide determinant to be the major target of the Abs. Mutations were clustered in the complementarity determining regions, suggesting that the Ab variable domains are products of adaptive affinity maturational processes. Infection of peripheral blood mononuclear cells by four diverse HIV-1 primary isolates was neutralized by the Fv fragments. The functional properties of the Fv clones point to the lupus repertoire as a unique source of Abs with possible utility in HIV immunotherapy.

*Abbreviations.* Ab, antibody; BSA, bovine serum albumin; CD4bs, CD4 binding site; PBMC, peripheral blood mononuclear cells

Identifying broadly neutralizing Abs to the envelope proteins of HIV has been a long-standing goal. Such Abs can help determine which antigenic determinants are suitable as targets for prophylactic vaccination. Moreover, the Abs themselves could potentially be applied for passive immunotherapy of AIDS if derived from a human source and available in monoclonal or recombinant form. Studies of the immune responses mounted in HIV-infected subjects and induced by experimental immunization with the envelope protein gp120 have indicated that the main epitopes recognized by Abs are the variable domains of the protein (1). These Abs generally do not neutralize primary HIV-1 isolates. Moreover, as the Ab-targeted epitopes are highly mutable, the Abs do not neutralize diverse HIV-1 strains found in different geographical regions and HIV-1 mutants that evolve in the course of infection in the same individual. Discontinuous peptide determinants constituting the site in gp120 responsible for recognizing host cell CD4 receptors, the CD4 binding site (CD4bs), tend to be conserved in diverse HIV-1 isolates. Abs to the CD4bs are formed only rarely, but certain Abs that recognize the CD4bs of monomeric gp120 have been prepared from sources such as Epstein Barr virus-transformed lymphocytes of HIV-1 infected individuals (2), Ab fragment libraries from an HIV-1 infected individual who remained free of AIDS for an extended period (3,4), and mice immunized with gp120 (5). Disappointingly, only a minority of the known Abs to the CD4bs display cross-clade neutralizing activity, a finding that has recently been attributed to conformational transitions occurring when native trimeric gp120 expressed on the viral surface dissociates into its monomeric form (6).

Systemic lupus erythematosus, an autoimmune disease, and HIV/AIDS, an immunodeficiency disease, coexist rarely. Although controlled epidemiological studies weighted for sexual habits and intravenous drug use remain to be carried out, several reports have discussed the strikingly small number of patients with concomitant lupus and HIV-1 infection, only twenty-nine cases worldwide (7-10). Interestingly, Abs to HIV-1 have been identified in the serum of lupus patients and mouse models of lupus (11-14). As there is no evidence of HIV-1 infection in the Ab-positive lupus subjects, the mechanism of Ab formation and their functional significance remains enigmatic. If the lupus repertoire expresses Abs to conserved gp120 determinants, it can serve as a useful source of anti-HIV Abs. These considerations impelled us to study M13 phage-



displayed Ab libraries prepared from the peripheral blood lymphocytes of HIV-negative lupus patients (15) as the source of HIV neutralizing Abs. We report the identification of affinity matured single chain Fv and light chain subunits capable of recognizing a conserved peptide determinant of gp120, residues 421-436. This determinant is a component of the CD4bs (16,17). The Fv clones neutralized the infectivity of diverse HIV-1 strains, raising interesting possibilities regarding their functional role and utility as anti-HIV reagents.

## Materials and Methods

### *Lupus Fv and L chain clones*

Preparation of the following phage displayed libraries has been described previously (15): (a) human L chains cloned in pCANTAB5his6 phagemid vector (pooled light chain cDNA from 3 lupus patients); (b) human single chain Fv constructs in pHEN2 phagemid vector ( $V_L$ -linker- $V_H$ ; linker, SS[GGGGS]<sub>2</sub>GGSA; pooled Fv cDNA from 2 lupus patients). Phages displaying the Fv libraries as p3-fusion proteins were packaged from TG1 cells using M13K07 helper phages ( $10^{13}$  particles) and selected by column chromatography on recombinant gp120 (strain SF2: Austral Biologicals) immobilized on Affigel-10 (Biorad) via Lys sidechains (1 ml settled gel; 47  $\mu$ g gp120/ml settled gel) using a pH 2.7 buffer for elution of bound phages (18). Similar procedures were applied to the L chain library except that the library had been preselected by binding to a phosphonate diester hapten (compound II in ref 15; this step enriches antibodies with nucleophilic activity, see ref 15 discussion section). Phage selection was conducted by "panning" on synthetic gp120(421-436) (KQIINMWQEVGKAMYA, corresponding to the consensus sequence of this determinant in clade B strains; 19). The peptide was immobilized (Nunc Maxisorp tubes; 10  $\mu$ g peptide/tube), blocked with 5% BSA, incubated with phages for 1 h and unbound phages removed by washing with 10 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4 containing 0.05% Tween-20 (PBS-Tween). Bound phages were eluted with 0.1 M glycine-HCl, pH 2.7 and immediately brought to neutral pH using 1 M Tris base (20). HB2151 cells were infected with the eluted phages, permitting expression of the antibody fragment in soluble form. Periplasmic extracts were prepared following induction with isopropyl- $\beta$ -D-thiogalactoside and recombinant proteins were purified by metal affinity chromatography to

electrophoretic homogeneity (the Ab fragments contain a his<sub>6</sub> tag; 15). SDS-polyacrylamide was on 8-25% Phast gels (Pharmacia), with identity of the proteins confirmed by immunoblotting using anti-cmyc Ab as described previously (15; the Ab fragments contain a ten residue c-myc peptide close to their C terminus).

### **ELISA**

Immobilized Cys-gp120(421-436) conjugated to bovine serum albumin (BSA; 10 mol peptide/mol BSA; 230 ng peptide equivalents/well) or full-length monomer gp120 (100 ng/well, MN strain, Immunodiagnostics Inc.) were used for ELISA (19). These were coated on Maxisorp 96-well microtiter plates (Nunc; 1 h), the plates blocked with 5% skim milk and incubated with recombinant Ab fragments in PBS-Tween containing 0.1% skimmed milk (1 h) in triplicate wells. Bound Ab fragments were detected using mouse anti-c-myc Ab (clone 9E10; 1:500 delipidated ascites) followed by peroxidase-conjugated goat anti-mouse Fc specific IgG (1:1000; Sigma) for color development. In competition ELISAs, the recombinant Abs were pretreated with diluent or competitor proteins (1  $\mu$ M; calmodulin, BSA, thyroglobulin; Sigma) for 1 h and then analyzed by ELISA.

### **HIV-1 neutralization**

HIV neutralization assays using PBMC hosts were carried out as described in (21,22) but with p24 quantification as the measure of infection. The following primary isolates of HIV-1 were obtained from the NIH AIDS Research and Reference Reagent Program: ZA009 (coreceptor CCR5, clade C), BR004 (coreceptor CCR5, clade C), Ug046 (coreceptor CXCR4, clade D) and SF-162 (coreceptor CCR5, clade B). HIV-1 primary isolate strain 23135 (coreceptor not known, clade B) was from Dr. Sandra Levine (Univ Southern California). Each virus stock was titrated in preliminary studies with each batch of donor PBMCs to determine the working dilution giving the optimum TCID<sub>50</sub>. The working dilution was adjusted to give p24 signal sufficient to be measured reproducibly in the linear range of the p24 assay after 4 days. The virus in RPMI was treated in quadruplicate with equal volumes of increasing concentrations (up to 50  $\mu$ g/ml) of metal affinity-purified Fv or L chain in PBS (1 h; TCID<sub>50</sub> for virus = 100). PHA-stimulated PBMCs from healthy human donors (0.25 million) were added to virus-Ab fragment mixtures and incubated for 3 days

(37°C), the cells washed twice with PBS and once with RPMI1640 (this removes potential interfering p24 from the original inoculum), incubated in fresh RPMI for 24 h, lysed with Triton X-100, and p24 in supernatants measured by an enzymeimmunoassay kit (Beckman Coulter HIV-1 p24 Antigen Assay Kit; linear range 50-3200 pg/ml). PBMC viability determination following incubation with Fv JL413, Fv JL427 or L chain SK18 (27 µg/ml, 72 h; incubations done exactly as in virus neutralization assays) was by staining with a mixture of acridine orange (2 µg/ml) and ethidium bromide (1 µg/ml) following by counting the viable cells (green fluorescence) using a hemocytometer and a UV microscope (23). Negative controls included the virus (strain ZA009) treated with: (a) diluent, (b) metal affinity purified extract of bacteria harboring pHEN2 vector (processed identically as recombinant Ab preparations); (c) light chain clone GG63 and SK161 (11 µg/ml, ref 15); and (d) Fv clones JL610 and JL611 (2.5 µg/ml). IgG clone b12 was kindly provided by Dr. Dennis Burton as a reference Ab.

## Results

### *Lupus anti-gp120 antibody fragments*

Single chain Fv constructs similar to those studied here reproduce faithfully the binding activity of full-length IgG Abs (e.g., 24). Previous reports have documented the antigen binding activity of L chain subunit independent of its H chain partner, albeit at reduced strengths compared to native Abs composed of both subunits (25,26). In the present study, two types of phage selections were carried out to enrich anti-HIV Ab fragments present in the lupus libraries: binding of Fv phages by full-length gp120, and of L chain phages by synthetic gp120(421-436). Monoclonal soluble Ab fragments obtained by expression of the selected phagemid DNA in HB2151 bacteria were screened for binding to gp120 and gp120(421-436) by ELISA. Fifty four percent and 26% of selected Fv and selected L chain clones were bound by full-length gp120, respectively, and 31% and 17% were bound by gp120(421-436) (Fig 1A). Except in the case of one Fv clone, binding of the two antigens was highly correlated (Fig 1B).

Two Fv clones (JL413, JL427) and one L chain clone (SK18, GenBank accession pending) were characterized further. Electrophoretically pure Fv and L chains from these clones displayed concentration-dependent gp120 and gp120(421-436) binding (Fig 2A-C). Competitive ELISA

studies indicated no significant reactivity of Fv JL427 with proteins unrelated to gp120(421-436) (Fig 2D).

Replacement mutations located in the CDRs of the V genes are usually interpreted to indicate adaptive maturation via somatic hypermutation processes (27). Comparison of the cDNA sequences of Fv JL413 and JL427 with their closest germline V gene counterparts revealed extensive replacement mutations in the regions contributed by the V<sub>L</sub> and V<sub>H</sub> genes (Table I; excluding the regions encoded by PCR primers or contributed by the D/J genes). The mutations tended to cluster in the complementarity determining regions. The ratios of replacement to silent mutations in the six CDRs for each Fv clone was greater than for the FRs. The V<sub>L</sub> domain of L chain clone SK18 contained 4 replacement mutations, with 1 replacement in the CDRs. Nonetheless, the replacement/silent mutation ratio for the CDRs remains greater than for FRs, as all of the silent mutations are located in the FRs. Additional alignments of the Fv and L chain clones with the closest germline V, (D) and J genes were attempted to assess junctional diversification. With introduction of gaps due to deletions and exclusion of FR4 sequence differences generated by the forward PCR primer [see Table 1 for germline J and D usage information; D gene usage for Fv JL427 V<sub>H</sub> is uncertain because of lack of discernable identity with germline D genes; the shortest available D segment, (D7-27, 11 nucleotides) was selected for alignment to obtain the most conservative diversification estimates]. The sequence analyses suggested extensive deletion and replacements as follows (number and gene identity in parentheses): Fv JL413 V<sub>L</sub> domain, 3 deletions (2V, 1J); Fv JL413 V<sub>H</sub> domain, 17 deletions (7V, 5D, 5J), 3 replacements (3D); Fv JL427 V<sub>L</sub> domain, 1 deletion (1V), 1 replacement (1J); Fv JL427 V<sub>H</sub> domain, 4 deletions (1V, 3D), 5 replacements (5J); L chain SK18 V<sub>L</sub> domain, 2 deletions (1V, 1J), 1 replacement (1J).

#### ***Cross-clade HIV-1 neutralization***

HIV-1 infection studies were done using PHA-stimulated peripheral blood mononuclear cells (PBMCs), with measurement of p24 antigen serving as the index of the level of infection (21,22). Progressively increasing neutralization of strain ZA009 by increasing concentrations of Fv JL413, Fv JL427 and light chain SK18 was observed (Fig 3). In control studies, no loss of HIV infectivity

(strain ZA009, clade C) was evident in the presence of identically purified Fv and L chains from several irrelevant clones (control Fv clone JL610 and L chain clone GG63 shown in Fig 3; not

shown, control Fv clone JL611, 2.5 µg/ml; control L chain clone SK161, 11 µg/ml) and the purified extract of bacteria harboring vector devoid of antibody inserts (15). In each case, p24 levels were statistically indistinguishable from virus control wells treated with PBS ( $p > 0.05$ , Student's t-test). At the aforementioned concentrations of the control clones, >75% neutralization of ZA009 strain was consistently observed in the presence of Fv JL413, Fv JL427 and L chain SK18. Inhibition of infection was not due to a cytotoxic effect, as no loss of cell viability was observed following incubation with Ab fragments in the absence of HIV (23). PBMC viability after treatment with diluent or the Ab fragments (Fv JL413, Fv JL427 or L chain SK18, 27 µg/ml) was  $81.2 \pm 2.8\%$  and  $74.8 - 80.9\%$ , respectively (150-200 cells counted). Consistent with the conserved nature of the antigenic recognition site (residues 421-436 of gp120), dose-dependent neutralization of each of the primary HIV-1 isolates by the purified Fv clones was observed (Table II). The infectivity of strains drawn from three distinct clades (B, C and D) was inhibited by the Fv constructs, suggesting broadly reactive neutralizing activity. Strains ZA009, SF-162 and BR004 utilize coreceptor CCR5, and clade D strain Ug046, coreceptor CXCR4. The L chain clone neutralized two of the three strains analyzed. Assays using independent preparations of the Ab fragments reproducibly indicated dose-dependent neutralization [strain ZA009,  $N=3$ ,  $IC_{50}$  for L chain SK18 L chain, Fv JL413 Fv and Fv JL427 in µg/ml:  $0.4 \pm 0.3$ ,  $0.2 \pm 0.1$  and  $0.3 \pm 0.1$  (s.d.), respectively; strain Ug046,  $N=2$ ,  $IC_{50}$  for L chain SK18 and Fv JL413: 11.6-13.5 and 2.1-5.5 µg/ml, respectively]. Side-by-side comparisons suggested that the neutralizing potency of monovalent Fv JL413 was comparable to bivalent IgG b12, a broadly neutralizing Ab to the CD4bs (28) (Fig 3).

The utility of binding to synthetic gp120(421-436) as a means for identifying HIV-neutralizing antibody fragments from the lupus repertoire is evident from these results. However, the binding data do not fully predict neutralization efficacy. For instance, L chain clone SK18 bound the antigens at considerably lower levels than the Fv clones (>2.7-fold for gp120(421-436) and >3.0-fold for gp120, Fig 2), but strains ZA009 and Ug046 were neutralized with similar potencies by all three Ab fragments (Table II). Differences in strain-reactivity of the individual Ab fragments are also evident. This is best exemplified by the L chain – no neutralization of strain 23135 was observed, whereas strains ZA009 and Ug046 were neutralized potently.

## Discussion

The lupus Fv clones described here bind a comparatively conserved constituent of the CD4bs, i.e., residues 421-436, and they display potent, cross-clade neutralization of primary HIV isolates dependent on R5 and X4 coreceptors. Polyclonal Abs to synthetic gp120(421-436) have been described previously in the serum of lupus patients and MRL/lpr mice, which spontaneously develop lupus-like symptoms (11,14). As the Fv clones were selected by binding to full-length gp120, their ability to recognize gp120(421-436) suggests this determinant to be the major epitope recognized by lupus Abs. Conversely, as the L chain clones were originally selected by binding to gp120(421-436), their reactivity with full-length gp120 indicates the ability of the synthetic peptide to assume a conformation similar to the cognate determinant expressed in full-length gp120. Promiscuous antigen binding activity patterns by Abs containing minimally mutated V domains have been described in previous studies (e.g., 29). These properties contrast with the gp120 binding Fv clones described here, in that extensive mutations were identified in the V domains and the binding activity was specific for gp120. Broad implications of the data reported here are: **(a)** The Abs may help dissect the antigenic structure of the CD4bs to identify important components susceptible to protective humoral immune response; **(b)** If the Abs fulfill certain additional criteria regarding the breadth, potency and ability to neutralize HIV-1 infection *in vivo*, they may serve as prototypes for the development of immunotherapeutic Abs; and **(c)** The detection of HIV neutralizing Fv clones in the library taken together with previous serum Ab studies (11,14) offers a rational explanation for the infrequent incidence of AIDS in lupus patients.

Structural analyses have suggested that certain contact residues in the complex of gp120 and CD4 are contributed by residues 421-436 (16,17). These residues are comparatively conserved in different HIV strains, presumably because CD4 binding is essential to maintain viral infectivity. Sequence polymorphisms in determinant 421-436 are located primarily at residues 429 and 432 (assessed by comparing all available sequences of this determinant in the Los Alamos database; 384 HIV-1 strains, clades A, B, C, D, F, G, H, J, U, N and O as well as the CRF and CPZ classifications; 40.7% and 42.0% of the strains contain the consensus residues at these positions; remaining positions are occupied by the consensus residues in 82.7 – 99.5% of the

strains). Fine structural differences in the CD4bs may result from the sequence polymorphisms with important consequences in regard to neutralizing activity. Full assessment of the extent to which the neutralizing activity of lupus Abs is tolerant to sequence divergence requires study of a large panel of HIV isolates containing defined polymorphisms in residues 421-436. Some initial conclusions are possible, however. The two Fv clones effectively neutralized strains ZA009 as well as Ug046 despite the differing identity of the residue at position 429 (Lys and Gly, respectively). Similarly, binding of the Ab fragments to synthetic peptide determinant 421-436 derived from strain SF2 was generally correlated with that to full-length gp120 from strain MN, despite the sequence difference at position 429 (Glu and Lys, respectively). Evidently, the Ab binding and neutralizing activities can be maintained despite certain sequence polymorphisms.

In addition to cross-clade HIV-1 neutralizing activity, high level potency is desirable for consideration of Abs in immunotherapy applications. Neutralizing potencies of the Fv clones compare favorably with the monovalent Fab fragments and bivalent IgG Abs proposed as candidates for HIV immunotherapy (e.g., 4). Further gains may be realized by recloning monovalent lupus Fv clones as bivalent IgG. The neutralizing potency of a monovalent Fab b12 is reportedly increased by 400-fold by recloning as a bivalent IgG, presumably due to increased avidity effects (28). Ab catalyzed gp120 digestion is another potential route towards enhanced potency, particularly in regard to the L chain subunit. Ab fragments with the ability to cleave peptide bond surrogates have been identified in the lupus phage libraries utilized in the present study (15) and two Ab L chains from other sources have previously been shown to cleave HIV coat proteins (14,30,31). Evidence that lupus Ab fragments express the correct epitope-specificity, i.e., recognition of determinant 421-436, suggests the feasibility of directing the catalytic function to this region of gp120. No cleavage of gp120 by the Ab fragments reported here was detected (assayed as in ref 14; data not shown), but phage selection conducted with a covalently reactive peptide analog of determinant 421-436 (31) have permitted identification of a gp120 cleaving L chain (Paul, S. and coworkers, to be published elsewhere).

The ability to bind the linear synthetic determinant 421-436 sets lupus Ab fragments apart from CD4bs-directed Abs described previously, the antigen binding activity of which depends on recognition of discontinuous segments of the CD4bs. The broad viral neutralizing behavior of the



lupus Abs is another distinctive property. Based on the thermodynamic parameters for binding of various Abs, a recent report has deduced conformational differences between the trimeric CD4bs of gp120 on the viral surface and the corresponding site in monomer gp120 (6). This helps explain the lack of broad HIV neutralizing activity of Abs that bind the CD4bs of monomer gp120 (e.g., 2). The functional properties of lupus Fv fragments compared to Abs formed in HIV-1 infection probably derive from unique immunological selection processes. The Fv mutational patterns (Table II) taken together with apparent absence of binding to unrelated proteins by Fv clone JL427 suggest that the lupus library can serve as a source of HIV-specific neutralizing Abs. It is difficult to attribute the HIV neutralization to cross-reactivity of anti-self Abs, as there are no known sequence identities between determinant 421-436 and human proteins, although other regions of gp120 express limited homologies with certain self antigens (33,34). We are left with the hypothesis that expression of endogenous retroviral gene products expressing structural similarity to the CD4bs may drive the synthesis of HIV Abs in lupus patients. The presence of retroviral gene sequences in the genome of uninfected humans is well known, and links between expression of such sequences and autoimmune disease have been discussed previously (35,36).

A previous study demonstrated the neutralization of a lab-adapted and a primary isolate of HIV-1 by polyclonal Abs present in sera of patients with mixed connective tissue disease and attributed the neutralizing activity to Abs that recognize the V3 loop of gp120 (37). Unfortunately, use of serum Abs precludes unambiguous assignment of the neutralizing activity to individual components in polyclonal mixtures. As rare Abs can be readily isolated by phage display methods, the present study does not allow inferences about the relative ability of lupus patients and non-autoimmune subjects to produce Abs directed to gp120(421-436). However, serum Abs capable of binding this determinant have been observed in human and murine lupus but not in control non-autoimmune subjects (11, 14), suggesting that production of these Abs is not a generalized phenomenon. These reports, taken together with the HIV-1 neutralizing activity of recombinant lupus Ab fragments in the present study, raise the possibility of a protective role of the Abs in defense against HIV. The presence of the Abs may help explain clinical observations of the rare coexistence of lupus and AIDS. Conversely, certain reports discuss clinical

amelioration of human lupus occurring upon the onset of viral immunodeficiency disease (9). In a murine experimental model, the symptoms of lupus are alleviated by induction of retroviral infection (37).

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## LEGENDS

**Fig 1: Selection of phage anti-gp120 antibody fragments from lupus libraries (A); and, correlated binding of lupus antibody fragments to full-length gp120 and synthetic gp120(421-436) (B).** Shown are ELISA values for Fv and L chain clones fractionated by prior binding of phage particles to immobilized gp120 and synthetic gp120, respectively (selected clones) or picked randomly from the unfractionated source libraries (unselected clones). N= number of independent clones analyzed. **A, Top**, Immobilized synthetic gp120(421-436) conjugated to bovine serum albumin. **A, bottom**, Immobilized full-length gp120. **B**, Plotted are selected Fv and L chain clones displaying A490 > 0.3 in Fig 1. Fv clones shown are: JL409, JL413, JL437 (●) and JL427 (■). L chain clones SK18, SK45, SK41, SK51 (▲).  $P = 0.0004$  for regression line (computed by excluding Fv JL427;  $r = 0.24$ ,  $P = 0.15$  with inclusion of this Fv). Data are corrected for binding by periplasmic extracts of bacteria harboring the vector without antibody insert [pHEN2 vector; A490 0.10 and 0.14 for gp120(421-436) and gp120 binding, respectively]. Recombinant antibody expression determined for 10 clones was  $1.95 \pm 0.51$  (s.e.m.) mg/liter bacterial culture. Differences in binding of gp120(421-436) and full-length gp120 by different clones observed by ELISA were not due to variations of the expression level ( $r^2$  0.10 and 0.17, respectively,  $P > 0.24$ ). PCR amplification using antibody framework primers confirmed the presence Fv and L chain inserts in the phagemid DNA (~0.7 kb; ref 15).

**Fig 2. Concentration-dependent binding of immobilized gp120(421-436) (■) and full-length monomer gp120 (●) by lupus antibody fragment (A-C) and specificity of binding to immobilized gp120(421-436) (D).** Recombinant proteins purified by metal affinity chromatography (13). **D**, Fv JL427 (46 µg/ml) treated with equal volume of soluble gp120(421-436), bovine serum albumin (BSA), thyroglobulin (Tg) and calmodulin (CaM) competitors (1 µM). **Insets**, silver stained SDS-polyacrylamide electrophoresis gels (8-25%) showing 27 kD purified antibody fragments purified (right lane in each panel) and marker proteins (left lane; from top to bottom, 94, 67, 43, 30, 20, 14 kD; Pharmacia).

**Fig 3. Concentration-dependent HIV-1 neutralization by lupus antibody fragments. A & B**, HIV-1 strain ZA009 (clade C), host cells PBMCs from healthy human donors. **C**, HIV-1



strain BR004 (clade C), host cells PBMCs from healthy human donors. See Table II legend for method and curve fitting procedure. Values are percent of p24 concentrations in culture wells containing HIV treated with PBS instead of Abs. Clones L chain SK18, Fv JL413, Fv JL427 and IgG b12: 4 independent culture replicates analyzed individually for p24 concentration (means  $\pm$  sem). Clones L chain GG61 and Fv JL610: pooled supernatants from 4 culture wells.

|             | L chain SK-18 | Fv JL-413 |                       | Fv JL-427  |                    |
|-------------|---------------|-----------|-----------------------|------------|--------------------|
|             |               | VL        | VH                    | VL         | VH                 |
| Family      | I             | I         | IV                    | I          | V                  |
| Subgroup    | I             | I         | II                    | I          | III                |
| Germline    | 02/012, Jk1   | L5, Jk4   | VH4-59,<br>D2-15, JH5 | V1-17, JL3 | VH3-48,<br>D*, JH6 |
| Counterpart |               |           |                       |            |                    |
| Mutations   |               |           |                       |            |                    |
| R           | 4             | 9         | 4                     | 14         | 13                 |
| S           | 3             | 3         | 3                     | 8          | 2                  |
| R/S CDRs    | 1/0           | 6/0       | 3/1                   | 9/3        | 8/1                |
| R/S FRs     | 3/3           | 3/3       | 1/2                   | 5/5        | 5/1                |

**Table I. Lupus antibody characteristics deduced from V domain sequences.** R – Replacement; S – Silent; D\* – germline D gene unassignable. Germline counterparts identified from <http://www.ncbi.nlm.nih.gov/igblast> (39,40). V gene mutation counts restricted to residues 8-95, V<sub>L</sub> domain, L chain SK18 (linear numbering); 8-94 and 8-91, V<sub>L</sub> and V<sub>H</sub> domains of Fv JL413, respectively; 8-120 and 8-98, V<sub>L</sub> and V<sub>H</sub> domains, Fv JL427, respectively. These residues correspond to lengths of germline V genes identified in lupus clones. FR1 residues 1-7 excluded because these are encoded by PCR back primers. Family and subgroup assignment from <http://immuno.bme.nwu.edu/>. cDNA sequences of Ab V domains determined in the 5' and 3' directions were identical.

| Ab           | B, 23135 |                  | B, SF-162 |                  | C, ZA009 |                  | C, BR004 |                 | D, Ug046         |                  |
|--------------|----------|------------------|-----------|------------------|----------|------------------|----------|-----------------|------------------|------------------|
|              | IC50     | IC90             | IC50      | IC90             | IC50     | IC90             | IC50     | IC90            | IC50             | IC90             |
| SK18 L chain | >54      | --               | nt        | nt               | 0.1      | 2.3 (0.7, 1.00)  | nt       | 13.5            | 18.8 (6.6, 1.00) |                  |
| JL413 Fv     | 25.6     | 44.6 (4.2, 0.99) | 2.2       | 97.7 (0.4, 0.99) | 0.1      | 16.1 (0.5, 1.00) | 0.1      | 2.8 (0.7, 0.98) | 1.0              | 10.0 (1.0, 0.97) |
| JL427 Fv     | 22.4     | 36.7 (4.5, 0.97) | nt        | nt               | 0.3      | 5.7 (0.7, 1.00)  | nt       | nt              | 5.5              | 12.7 (2.6, 0.96) |
| b12 IgG      | nt       | nt               | 1.1       | 14.1 (0.9, 0.94) | nt       | nt               | 9.0      | 333 (0.6, 0.89) | nt               | nt               |

**Table II. HIV-1 neutralization by lupus Ab fragments.** Values are reported as  $\mu\text{g}$  Ab fragment/ml yielding 50% (IC50) and 90% (IC90) neutralization. In parentheses are, respectively, Hill slopes and squared correlation coefficients for curves fitted to the equation: % HIV neutralization =  $100\% / [1 + 10^{((\log(\text{IC50} - \text{Ab concentration}) \times \text{Hill slope}))}]$ . Curves forced through origin (zero neutralization); Hill slope value held as a variable parameter. nt, not tested.

Figure 1

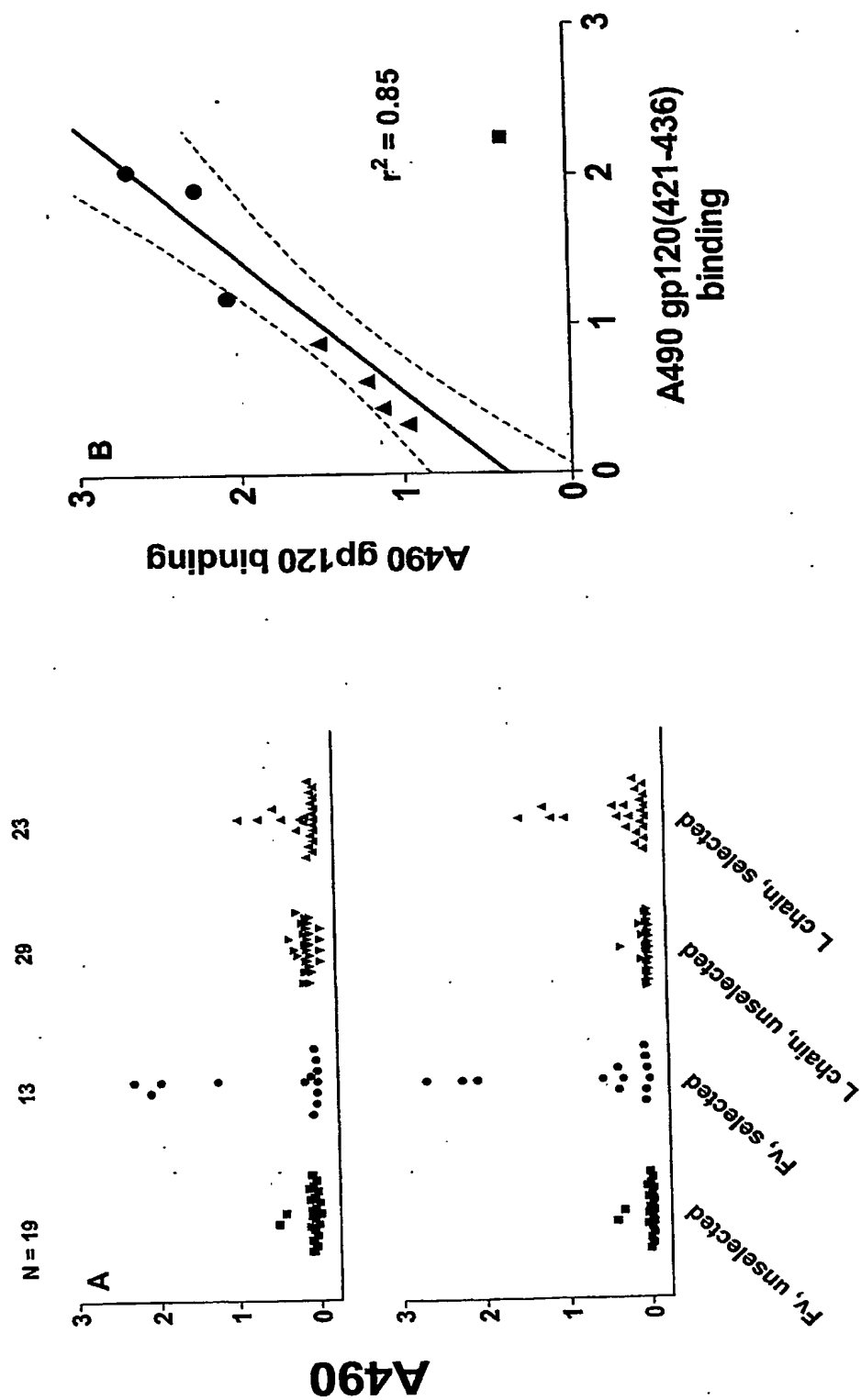


Figure 2

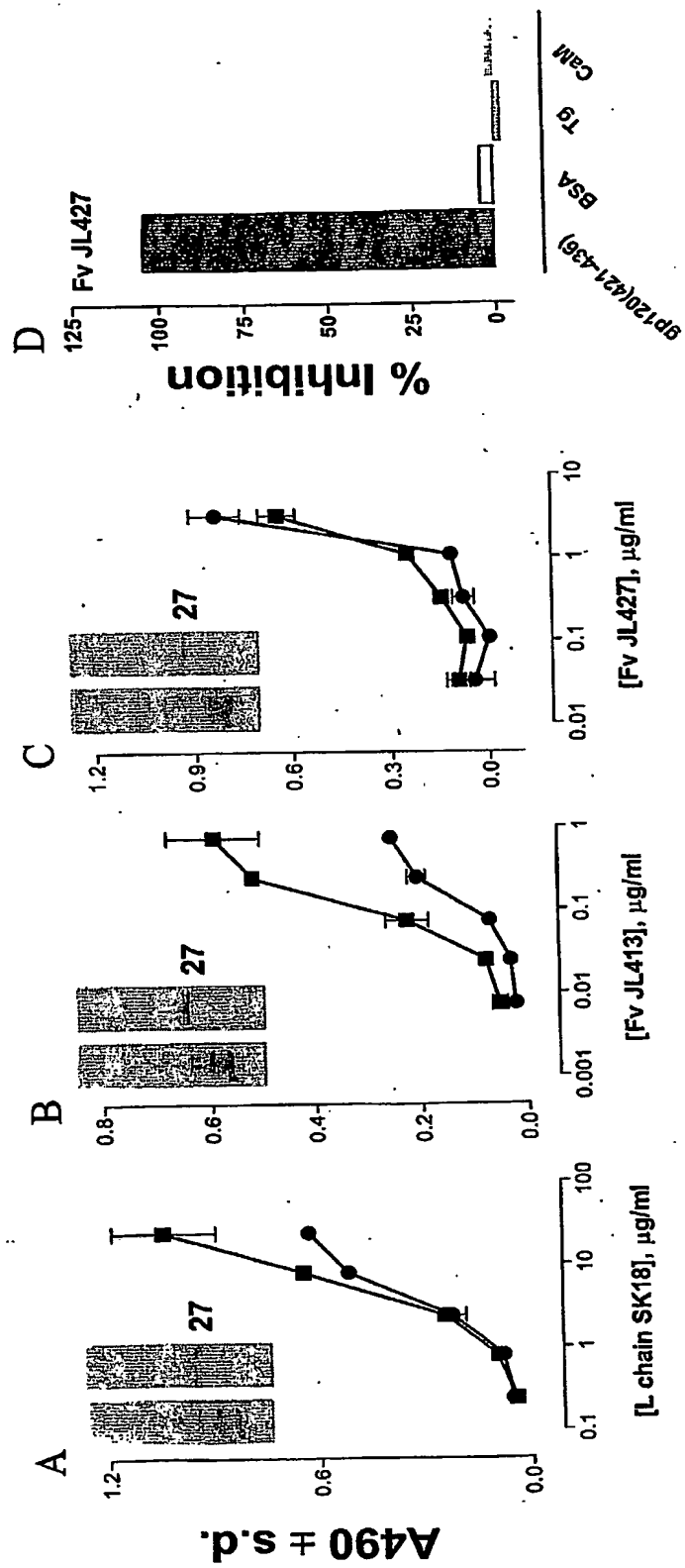
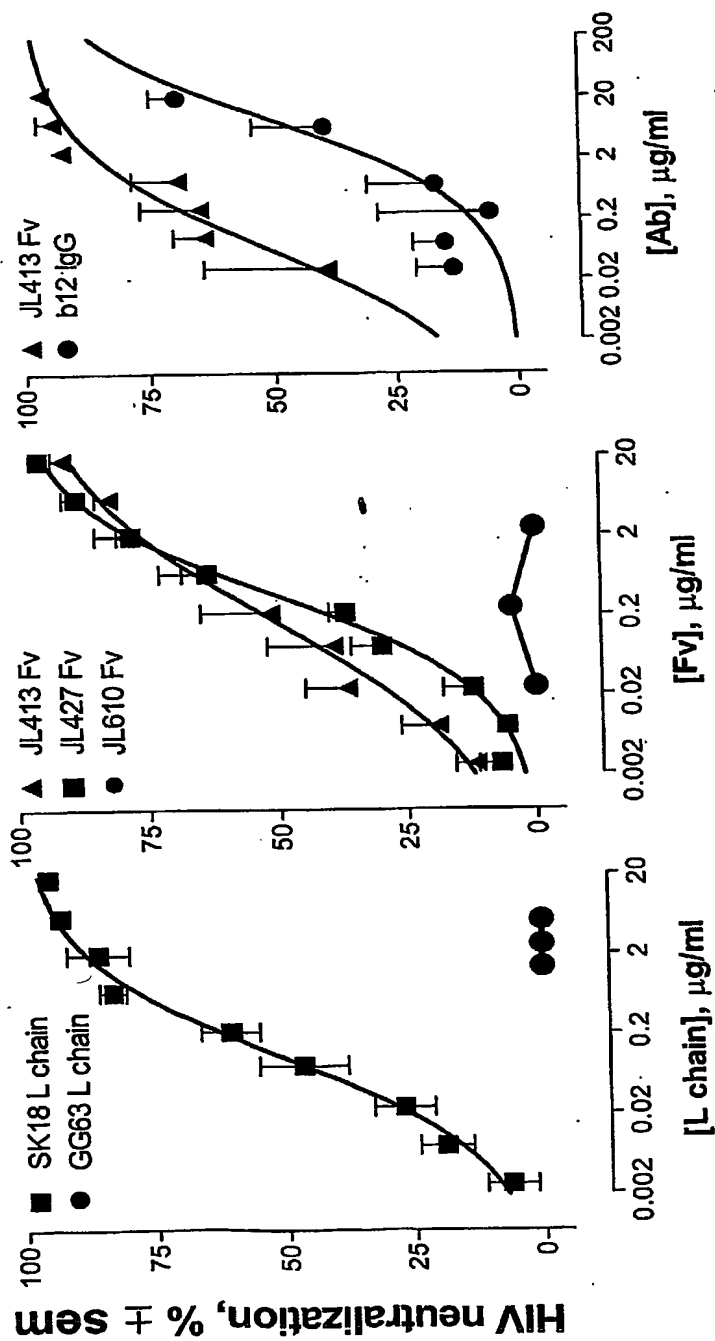


Figure 3



Linearly numbered nucleotide (N) and amino acid (R) sequences of lupus V domains shown with no numbering breaks at V, (D), J junctions. Germline counterpart V, (D), J genes are numbered individually, starting with R/N# 1 for each gene. Numbers above and below sequences pertain to lupus clones and germline counterparts, respectively. Overlined (lupus V domains) and underlined (germline counterparts) sequences are complementary determining regions (CDRs). Fv JL413 CDR3H assignment is ambiguous (dotted overline; see alternate alignments 1 and 2, pages 4 and 5). Dots and dashes represent nucleotide and amino acid identities, respectively. Solid vertical lines demarcate V, D and J genes. V gene residues analyzed in Table 1 are C terminal to the broken vertical lines. At the C termini of light chain and the Fv clones are located the complete CL domain and CH1 residues and the N terminal 9 residues of CH1, respectively, followed by the his6 and c-myc tags (HHHHHHGAAEQKLISEEDLN; catcatcatcaccatcacggggcgcgagaacaaaaactcatctcagaagaggatctgaat). A linker sequence links the C terminus of the Fv VL domain to the N terminus of the VH domain (SSGGGSGGGGSGGSA; tcgagtgggtggaggcggttcaggcgagggtggctctggcggtagtgca). See Fv JL413 VH for discussion of alternate junctional regions alignments.

| (A) <u>Light chain SK18 VL domain</u> |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#                                    | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |     |
| N#                                    | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |     |
| SK18                                  | ASP | ILE | GLN | MET | THR | GLN | SER | PRO | SER | SER | LEU | SER | ALA | SER | VAL | GLY | ASP | ARG | VAL | THR |
| SK18                                  | gac | atc | cag | atg | acc | cag | tct | cca | tcc | tcc | ctg | tct | gca | tct | gtg | gga | gac | aga | gtc | acc |
| 02/012                                | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 02/012                                | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ... | ... | ... | ... | --- | --- |
| N#                                    | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |     |
| R#                                    | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |     |

|        |     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|--------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#     | 21  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 40  |     |     |     |     |
| N#     | 61  | CDR1 |     |     |     |     |     |     |     |     |     |     |     |     |     | 120 |     |     |     |     |
| SK18   | VAL | THR  | CYS | ARG | ALA | SER | GLN | SER | ILE | SER | SER | TYR | LEU | ASN | TRP | TYR | GLN | GLN | GLN | PRO |
| SK18   | gtc | act  | tgc | cgg | gca | agt | cag | agc | att | agc | agc | tat | tta | aat | tgg | tat | cag | cag | caa | cca |
| 02/012 | a.. | ...  | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | a.. | --- |
| 02/012 | ILE | ---  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#     | 61  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 120 |     |     |     |     |
| R#     | 21  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 40  |     |     |     |     |

|        |     |      |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|--------|-----|------|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#     | 41  |      |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     | 60  |     |     |
| N#     | 121 | CDR2 |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     | 180 |     |     |
| SK18   | GLY | LYS  | ALA | PRO | LYS  | LEU | LEU | ILE | TYR | ALA | ALA | SER | SER | LEU | GLN | SER | GLY | VAL | PRO | SER |
| SK18   | ggg | aag  | gcc | cct | aaa  | ctc | ctg | atc | tat | gct | gca | tcc | agt | ttg | caa | agt | ggg | gtc | cca | tca |
| 02/012 | ... | ...a | ... | ... | ...g | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 02/012 | --- | ---  | --- | --- | ---  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#     | 121 |      |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     | 180 |     |     |
| R#     | 41  |      |     |     |      |     |     |     |     |     |     |     |     |     |     |     |     | 60  |     |     |

|        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#     | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  |     |
| N#     | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |     |
| SK18   | ARG | PHE | SER | GLY | SER | GLY | SER | GLY | THR | ASP | PHE | THR | LEU | THR | ILE | SER | SER | LEU | GLN | PRO |
| SK18   | agg | ttc | agt | ggc | agt | gga | tct | ggg | aca | gat | ttc | act | ctc | acc | atc | agc | agt | ctg | caa | cct |
| 02/012 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 02/012 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#     | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |     |
| R#     | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  |     |

|        |                                 |                         |     |     |     |  |  |  |  |  |  |  |  |  |  |          |          |
|--------|---------------------------------|-------------------------|-----|-----|-----|--|--|--|--|--|--|--|--|--|--|----------|----------|
| R#     | 81                              |                         |     |     |     |  |  |  |  |  |  |  |  |  |  | 95       |          |
| N#     | 241                             | CDR3                    |     |     |     |  |  |  |  |  |  |  |  |  |  | 285      |          |
| SK18   | GLU ASP PHE ALA THR TYR PHE CYS | GLN GLN SER TYR SER ILE | PRO | dd  | d   |  |  |  |  |  |  |  |  |  |  |          |          |
| SK18   | gaa gat ttt gca act tac ttc tgt | caa cag agt tac agt atc | cct | dd  | d   |  |  |  |  |  |  |  |  |  |  |          |          |
| 02/012 | ... .. .                        | ... .. .                | .c. | cc  | g   |  |  |  |  |  |  |  |  |  |  |          |          |
| 02/012 | ... .. .                        | ... .. .                | THR | --- | PRO |  |  |  |  |  |  |  |  |  |  |          |          |
| N#     | 241                             |                         |     |     |     |  |  |  |  |  |  |  |  |  |  | 285      |          |
| R#     | 81                              |                         |     |     |     |  |  |  |  |  |  |  |  |  |  | 95       |          |
|        |                                 |                         |     |     |     |  |  |  |  |  |  |  |  |  |  | ← V gene | J gene → |

|          |                    |   |        |  |  |  |  |  |  |  |  |  |     |
|----------|--------------------|---|--------|--|--|--|--|--|--|--|--|--|-----|
| R#       | 96                 |   |        |  |  |  |  |  |  |  |  |  | 107 |
| N#       | <u>286</u>         |   |        |  |  |  |  |  |  |  |  |  | 321 |
| SK18     | ddd ARG THR        | PHE GLY GLN GLY THR LYS VAL GLU ILE LYS | d      |  |  |  |  |  |  |  |  |  |     |
| SK18     | ddd cga acg        | ttc ggc caa ggg acc aag gtg gaa atc aaa | d<br>c |  |  |  |  |  |  |  |  |  |     |
| Jk1      | tgg .cg ...        | ... ..                                  |        |  |  |  |  |  |  |  |  |  |     |
| Jk1      | <u>TRP PRO ---</u> | ---                                     |        |  |  |  |  |  |  |  |  |  |     |
| N#       | 5                  |   | 40     |  |  |  |  |  |  |  |  |  |     |
| R#       | 2                  |   | 13     |  |  |  |  |  |  |  |  |  |     |
| J gene → |                    |   |        |  |  |  |  |  |  |  |  |  |     |



## (B) Fv JL413

VL domain  
R# 1 20  
N# 1 60  
JL413 VL ASP VAL VAL MET THR GLN SER PRO SER SER VAL SER ALA SER VAL GLY ASP ARG VAL THR  
JL413 VL gat gtt gtg atg act cag tct cca tct tcc gtg tct gca tct gta gga gac aga gtc acc  
L5 ..c a.c ca. ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c  
L5 --- ILE GLN --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  
N# 1 V gene → 60  
R# 1 20

R# 21 40 120  
N# 61 CDR1  
JL413 VL ILE THR CYS ARG ALA SER GLN GLY ILE GLY ASN TRP LEU ALA TRP TYR GLN GLN LYS PRO  
JL413 VL atc act tgt cgg gcg agt cag ggt att ggc aac tgg tta gcc tgg tat cag cag aaa cct  
L5 ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c  
L5 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  
N# 61 120  
R# 21 40

R# 41 60 180  
N# 121 CDR2  
JL413 VL GLY LYS ALA HIS ASN LEU LEU ILE TYR GLY ALA SER SER LEU GLN SER GLY VAL PRO SER  
JL413 VL ggg aaa gcc cat aac ctc ctg atc tat ggt gca tcc agt ttg caa agt ggg gtc cca tca  
L5 ... ..c ... ..g ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c  
L5 --- --- --- PRO LYS --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  
N# 121 180  
R# 41 60

R# 61 80 240  
N# 181  
JL413 VL ARG PHE SER GLY SER GLY SER GLY THR ASP PHE THR LEU THR ILE SER SER LEU GLN PRO  
JL413 VL agg ttc agc ggc agt gga tct ggg aca gac ttc act ctc acc atc agc agc ctg cag cct  
L5 ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c  
L5 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  
N# 181 240  
R# 61 80

R# 81 94  
N# 241 CDR3 282  
JL413 VL GLU ASP SER ALA THR TYR TYR CYS GLN GLN ALA LEU VAL GLY ddd dd d  
JL413 VL gaa gac tct gca act tac tat tgt caa cag gct ctc gtg ggt ddd dd d  
L5 ... ..t .t. ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c ... ..c  
L5 --- --- PHE --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  
N# 241 282  
R# 81 94  
← V gene | J gene →

|          |   |     |
|----------|---|-----|
| R#       | 95  | 105 |
| N#       | 283   | 315 |
| JL413 VL | ddd THR PHE GLY GLY GLY THR LYS VAL GLU ILE LYS ARG |     |
| JL413 VL | ddd act ttc ggc gga ggg acc aag gtg gag atc aaa cgc |     |
| Jk4      | ctc ... .. .dd                                      |     |
| Jk4      | LEU --- --- -dd                                     |     |
| N#       | 5   | 37  |
| R#       | 2   | 12  |

J gene→

|                                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Fv JL413 VH domain, alignment 1 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |
| R#                              | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| N#                              | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| JL413 VH                        | GLN | VAL | ASN | LEU | ARG | GLU | SER | GLY | PRO | GLY | LEU | VAL | LYS | PRO | SER | GLU | THR | LEU | SER | LEU |
| JL413 VH                        | cag | gtc | aac | tta | agg | gag | tct | ggc | cca | gga | ctg | gtg | aag | cct | tcg | gag | acc | ctg | tcc | ctc |
| VH4-59                          | ... | ..g | c.g | .t. | ca. | ... | ..g | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| VH4-59                          | --- | --- | GLN | --- | GLN | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#                              | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| R#                              | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |

|          |     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 21  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 40  |     |     |     |     |
| N#       | 61  | CDR1 |     |     |     |     |     |     |     |     |     |     |     |     |     | 120 |     |     |     |     |
| JL413 VH | THR | CYS  | THR | VAL | SER | GLY | GLY | PHE | ILE | SER | SER | TYR | TYR | TRP | SER | TRP | ILE | ARG | GLN | PRO |
| JL413 VH | acc | tgc  | act | gtc | tct | ggt | ggc | ttc | atc | agt | agt | tac | tac | tgg | agc | tgg | atc | cgg | cag | ccc |
| VH4-59   | ... | ...  | ... | ... | ... | ... | ... | .C. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| VH4-59   | --- | ---  | --- | --- | --- | --- | --- | SER | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#       | 61  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 120 |     |     |     |     |
| R#       | 21  |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 40  |     |     |     |     |

|          |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 41  |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     | 60  |     |     |     |
| N#       | 121 |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     | 180 |     |     |     |
| JL413 VH | PRO | GLY | LYS | GLY | LEU | GLU | TRP | ILE | GLY | CDR2 |     |     |     |     |     | ASN |     |     |     |     |
| JL413 VH | cca | ggg | aag | gga | ctg | gag | tgg | att | ggg | tth  | acc | tat | tac | agt | ggg | agc | acc | tac | tac | aac |
| VH4-59   | ... | ... | ... | ... | ... | ... | ... | ... | ... | a.   | t.  | ... | ... | ... | ... | ... | a.  | ... | ... | ... |
| VH4-59   | --- | --- | --- | --- | --- | --- | --- | --- | --- | TYR  | ILE | --- | --- | --- | --- | --- | ASN | --- | --- | --- |
| N#       | 121 |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     | 180 |     |     |     |
| R#       | 41  |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     | 60  |     |     |     |

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  |     |     |     |
| N#       | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |     |     |     |
| JL413 VH | PRO | SER | LEU | LYS | SER | ARG | VAL | THR | ILE | SER | VAL | ASP | THR | SER | LYS | ASN | GLN | PHE | SER | LEU |
| JL413 VH | cgc | tcg | ctc | aag | agt | cga | gtt | acc | ata | tca | gta | gac | acg | tct | aag | aac | cag | ttc | tcc | ctg |
| VH4-59   | ..C | ... | ... | ... | ... | ... | ..C | ... | ... | ... | ... | ... | ... | ..C | ... | ... | ... | ... | ... | ... |
| VH4-59   | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| N#       | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240 |     |     |     |
| R#       | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  |     |     |     |

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |  |  |  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|--|--|--|
| R#       | 81  |     |     |     |     |     |     |     |     |     |     | 91  |     |     |     |     |     |     |   |  |  |  |
| N#       | 241 |     |     |     |     |     |     |     |     |     |     | 273 |     |     |     |     |     |     |   |  |  |  |
| JL413 VH | LYS | LEU | SER | SER | VAL | THR | ALA | ALA | ASP | THR | ALA | ddd | ddd | ddd | ddd | ddd | ddd | dd  | d |  |  |  |
| JL413 VH | aag | ctg | agc | tct | gtg | acc | gct | gcg | gac | acg | gcc | ddd | ddd | ddd | ddd | ddd | ddd | dd  | d |  |  |  |
| VH4-59   | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | gtg | tat | tac | tgt | gcg | aga | ga  | a |  |  |  |
| VH4-59   | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | VAL | TYR | TYR | CYS | ALA | ARG | GLU |   |  |  |  |
| N#       | 241 |     |     |     |     |     |     |     |     |     |     | 273 |     |     |     |     |     |     |   |  |  |  |
| R#       | 81  |     |     |     |     |     |     |     |     |     |     | 91  |     |     |     |     |     |     |   |  |  |  |

← V gene | D gene →

D gene →

|          |    |            |            |            |            |            |     |     |     |     |     |     |     |     |     |     |     |    |     |
|----------|----|------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|
| R#       |    |            |            |            |            |            | 97  |     |     |     |     |     |     |     |     |     |     |    | 103 |
| N#       |    |            |            |            |            |            | 289 |     |     |     |     |     |     |     |     |     | 321 |    |     |
| JL413 VH | dd | ddd        | ddd        | ddd        | ddd        | ddd        | TRP | GLY | GLN | GLY | THR | LEU | VAL | THR | VAL | SER | SER |    |     |
| JL413 VH | dd | ddd        | ddd        | ddd        | ddd        | ddd        | tgg | ggc | cag | gga | acc | ctg | gtc | acc | gtc | tcc | tca |    |     |
| JH5      | ac | aac        | tgg        | ttc        | gac        | tcc        | ... | ... | ..a | ... | ... | ... | ... | ... | ... | ... | ... |    |     |
| JH5      |    | <u>ASN</u> | <u>Tyr</u> | <u>PHE</u> | <u>ASP</u> | <u>SER</u> | --  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |    |     |
| N#       |    |            |            |            |            |            | 18  |     |     |     |     |     |     |     |     |     |     | 50 |     |
| R#       |    |            |            |            |            |            | 6   |     |     |     |     |     |     |     |     |     |     | 16 |     |

J gene →

## Fv JL413 VH domain, alignment 2 (junctional region aligned according to referee suggestion)

R# 81 96 288  
 N# 241  
 JL413 VH LYS LEU SER SER VAL THR ALA ALA ASP THR ALA MET TYR CYS TYR CYS ddd dd d  
 JL413 VH aag ctg agc tct gtg acc gct gcg gac acg gcc atg tat tgc tac tgc ddd dd d  
 VH4-59 ... .. g.. ..a. .gt gcg aga ga a  
 VH4-59 --- --- --- --- --- --- --- --- --- VAL --- TYR CYS ALA ARG GLU  
 N# 241 273  
 R# 81 91  
 ← V gene | D gene →

JL413 VH ddd ddd ddd ddd ddd ddd ddd ddd ddd ddd  
 JL413 VH ddd ddd ddd ddd ddd ddd ddd ddd ddd ddd  
 D2-15 gga tat tgt agt ggt ggt agc tgc tac .c.  
 D2-15 GLY TYR CYS SER GLY GLY SER CYS TYR SER  
 N# 2 31  
 R# 1 10  
 D gene →

R# 97 103  
 N# 289 321  
 JL413 VH dd ddd ddd ddd ddd ddd TRP GLY GLN GLY THR LEU VAL THR VAL SER SER  
 JL413 VH dd ddd ddd ddd ddd ddd tgg ggc cag gga acc ctg gtc acc gtc tcc tca  
 JH5 ac aac tgg ttc gac tcc ... ..a ... ..  
 JH5 ASN TYR PHE ASP SER ... ..  
 N# 18 50  
 R# 6 16  
 J gene →

| Alignment | Nucleotides, #274-288 |           | Amino acids, #91-96 |           |
|-----------|-----------------------|-----------|---------------------|-----------|
|           | Replacements          | Deletions | Replacements        | Deletions |
| 1         | 7/15                  | 53        | 3/5                 | 17        |
| 2         | 7/15                  | 53        | 4/5                 | 17        |

VL domain

| VL C-100 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| N#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |
| JL427 VL | GLN | SER | VAL | LEU | THR | GLN | PRO | PRO | SER | VAL | SER | GLY | ALA | PRO | GLY | GLN | ARG | VAL | THR | ILE |
| JL427 VL | cag | tct | gtg | ttg | acg | cag | cgc | ccc | tca | gtg | tct | ggg | gcc | cca | ggg | cag | agg | gtc | acc | att |
| V1-17    | ... | ... | ... | c.. | ..t | ... | ..a | ... | ... | ..c | ... | ... | ... | a.. | ..c | ... | ... | ... | ... | ..c |
| V1-17    | --- | --- | --- | --- | --- | --- | --- | --- | --- | ALA | --- | --- | THR | --- | --- | --- | --- | --- | --- | --- |
| N#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| R#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |

|          |     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 21  | CDR1 |     |     |     |     |     |     |     |     |     |     |     | 120 | 40  |     |     |     |     |     |     |
| N#       | 61  | SER  | CYS | SER | GLY | SER | SER | SER | ASN | PHE | GLY | LEU | ASN | TYR | VAL | TYR | TRP | TYR | GLN | HIS | PHE |
| JL427 VL | SER | CYS  | SER | GLY | SER | SER | SER | ASN | PHE | GLY | LEU | ASN | TYR | VAL | TYR | TRP | TYR | GLN | HIS | PHE |     |
| JL427 VL | tct | tgt  | tct | gga | agc | agc | tcc | aac | ttc | ggc | ctt | aat | tat | gta | tac | tgg | tat | cag | cac | ttc |     |
| V1-17    | ... | ...  | ... | ... | ... | ... | ... | ... | a.. | ..a | ag. | ... | ... | ... | ... | ... | ..c | ... | ..g | c.. |     |
| V1-17    | --- | ---  | --- | --- | --- | --- | --- | --- | ILE | --- | SER | --- | --- | --- | --- | --- | --- | --- | GLN | LEU |     |
| N#       | 61  |      |     |     |     |     |     |     |     |     |     |     |     | 120 | 40  |     |     |     |     |     |     |
| R#       | 21  |      |     |     |     |     |     |     |     |     |     |     |     | 120 | 40  |     |     |     |     |     |     |

| R#       | 41  | CDR2 |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  | 180 |     |     |     |     |
|----------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| N#       | 121 | PRO  | GLY | THR | ALA | PRO | LYS | LEU | LEU | ILE | TYR | ARG | ASN | ASP | GLN | ARG | PRO | LEU | GLY | VAL | PRO |
| JL427 VL | cca | gga  | acg | gcc | ccc | aaa | ctc | ctc | atc | tat | agg | aat | gat | cag | cgg | ccc | tta | ggg | gtc | cct |     |
| JL427 VL | ... | ...  | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |     |
| V1-17    | ... | ...  | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |     |
| V1-17    | ... | ...  | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |     |
| N#       | 121 |      |     |     |     |     |     |     |     |     |     |     |     |     |     | 80  | 180 |     |     |     |     |
| R#       | 41  |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

[illegible]

| R#       | 101 |     |     |     |     |     |     |     |     |     |     | CDR3 |     |     |     | 300 |     |     |     | 120 |  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|--|
| N#       | 241 |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     |     |  |
| JL427 VL | SER | GLU | ASP | GLU | ALA | ASP | TYR | TYR | CYS | GLN | SER | TYR  | ASP | ASN | SER | LEU | SER | GLY | dd  | d   |  |
| JL427 VL | tcc | gag | gat | gag | gct | gat | tat | tac | tgc | cag | tcc | tat  | gac | aac | agc | ctg | agt | ggc | dd  | d   |  |
| V1-17    | ... | ... | ... | ... | ... | ... | ... | ... | ..t | gca | g.a | .gg  | .t  | g.. | ... | ... | ... | ..t | cc  | t   |  |
| V1-17    | ... | --- | --- | --- | --- | --- | --- | --- | --- | ALA | ALA | TRP  | --- | ASP | --- | --- | --- | --- | --- | PRO |  |
| N#       | 241 |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     | 300 |  |
| R#       | 101 |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |     |     |     | 120 |  |

← V gene | J gene →

9

[illegible]

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |
| N#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| JL427 VH | GLN | VAL | GLN | LEU | GLN | GLN | SER | GLY | GLY | GLY | LEU | VAL | GLN | PRO | GLY | ARG | SER | LEU | ARG | LEU |
| JL427 VH | cag | gta | cag | ctg | cag | cag | tca | ggg | gga | ggc | ttg | gta | cag | cct | ggg | agg | tcc | ctg | aga | ctc |
| VH3-48   | g.. | ..g | ... | ... | gt. | g.. | ..t | ... | ... | ... | ... | ... | ... | ... | ... | g.. | ... | ... | ... | ... |
| VH3-48   | GLU | --- | --- | --- | VAL | GLU | --- | --- | --- | --- | --- | --- | --- | --- | --- | GLY | --- | --- | --- | --- |
|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| N#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| R#       | 1   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |

|          |     |     |     |      |     |     |     |             |     |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----|-----|------|-----|-----|-----|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R#       | 21  |     |     |      |     |     |     |             |     |     |     |     |     |     |     |     |     |     |     | 40  |
| N#       | 61  |     |     |      |     |     |     |             |     |     |     |     |     |     |     |     |     |     |     | 120 |
| JL427 VH | SER | CYS | ALA | ALA  | SER | GLY | PHE | <u>CDR1</u> |     |     |     | GLY | MET | HIS | TRP | VAL | ARG | GLN | ALA |     |
| JL427 VH | tcc | tgt | gca | gcg  | tct | gga | ttc | acc         | ttc | agt | agc | tat | ggc | atg | cac | tgg | gtc | cgc | cag | gct |
| VH3-48   | ... | ... | ... | ...c | ... | ... | ... | ...         | ... | ... | ... | ... | a.. | ... | a.. | ... | ... | ... | ... |     |
| VH3-48   | --- | --- | --- | ---  | --- | --- | --- | ---         | --- | --- | --- | --- | SER | --- | ASN | --- | --- | --- | --- |     |
| N#       | 61  |     |     |      |     |     |     |             |     |     |     |     |     |     |     |     |     |     |     | 120 |
| R#       | 21  |     |     |      |     |     |     |             |     |     |     |     |     |     |     |     |     |     |     | 40  |

|          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |  |  |  |  |  |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|--|--|--|--|--|-----|
| R#       | 41  |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60   |     |     |     |     |  |  |  |  |  |     |
| N#       | 121 |     |     |     |     |     |     |     |     |     |     |     |     |     |     | CDR2 |     |     |     |     |  |  |  |  |  | 180 |
| JL427 VH | PRO | GLY | LYS | GLY | LEU | GLU | TRP | VAL | SER | TYR | ILE | GLY | ARG | SER | GLY | SER  | HIS | THR | ASN | TYR |  |  |  |  |  |     |
| JL427 VH | cca | ggg | aag | ggg | ctg | gag | tgg | gtt | tca | tac | att | ggt | aga | agt | ggt | agt  | cac | aca | aac | tac |  |  |  |  |  |     |
| VH3-48   | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | a.. | ..t | ... | a.. | ...  | ac. | .t. | t.. | ... |  |  |  |  |  |     |
| VH3-48   | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | SER | SER | --- | SER | ---  | THR | ILE | TYR | --- |  |  |  |  |  |     |
| N#       | 121 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |     |     |     |     |  |  |  |  |  | 180 |
| R#       | 41  |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60   |     |     |     |     |  |  |  |  |  |     |

| R#       | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80   |     |     |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|
| N#       | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240  |     |     |
| JL427 VH | ALA | ASP | SER | VAL | LYS | GLY | ARG | PHE | THR | ILE | SER | ARG | ASP | ASN | SER | LYS | ASN | THR  | LEU | TYR |
| JL427 VH | gca | gac | tcc | gtg | aag | ggc | cga | ttc | acc | atc | tcc | aga | gac | aat | tcc | aag | aac | acg  | ctg | tat |
| VH3-48   | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | g.. | ... | ... | t..a | ... | ... |
| VH3-48   | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ALA | --- | --- | SER  | --- | --- |
| N#       | 181 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 240  |     |     |
| R#       | 61  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 80   |     |     |

[illegible]



D gene →

| R#       | 99       | CDR3 |     |     |     |     |     |     |     |     |     |     |     |     |     | 118 | 353 |     |     |     |     |
|----------|----------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| N#       | 294      | GLY  | LEU | PRO | ASN | TYR | GLY | MET | ASP | ILE | TRP | GLY | GLN | GLY | THR | THR | VAL | THR | VAL | SER | SER |
| JL427 VH | ggg      | ctg  | cca | aac | tac | ggt | atg | gac | atc | tgg | ggc | caa | ggg | acc | acg | gtc | acc | gtc | tcc | tca |     |
| JL427 VH | .ac      | tac  | tac | tac | ... | ... | ... | ... | g.. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |     |
| JH6      | ASP      | TYR  | TYR | TYR | --- | --- | --- | --- | VAL | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |     |
| N#       | 1        |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 60  |
| R#       | 1        |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 20  |
|          | J gene → |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

**Supplementary information, attachment 2:** Amino acid sequences of twelve VH domains of Fv clones isolated from the lupus phage library. Clones 78 and 129 isolated by binding to immobilized prothrombin (unpublished data). Clones SP7D2, SP1A2 and SP8C5 isolated by binding to immobilized extracellular domain of epidermal growth factor receptor (Genbank accession# AF329456, AF329457, AF329458, respectively; Planque et al. FASEB J. 17, 136-143 (2003)). Clones DM319, DM412, DM506, JB14, JB43 and JB48 identified by binding to vasoactive intestinal polypeptide (Genbank accession# AF416909, AF416907, AF509586, AF509587, AF416908, AF416910, AF416909, respectively; Bangale et al. Peptides 23, 2251-2257 (2002); Bangale et al. FASEB J. In press, attached manuscript 3). No truncation of FR3 is evident in any clones. Unambiguous CDR3 segments ranging in length from 9 - 18 amino acids are evident in the clones.

Fv 78  
QLVPSGGGLVQPGSLRLSCAASGFTFDYAMHWVRQAPGKGLEWVSGISWNSGSGIYADSVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCAKDLSSGWIPPRKYYGMDXWGQGTITVTVSS

Fv 129  
QLQESGGGLVQPGSLRLSCAASGFAFSSYAMHWVRQAPGKGLEWVAISVDGSKKYYADSVKGRFTISRDNKNTLYLQMSLRAEDTAVYYCARDLYYSKYYCYGMDVWGQGTITVTVSS

Fv SP7D2  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARASGVSYGDFDYGQGTITVTVSS

Fv SP1A2  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv SP8C5  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCAIPIGLRRAEDYWGQGTITVTVSS

Fv DM319  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv DM412  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv DM408  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv DM506  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv JB14  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv JB43  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

Fv JB48  
LQVQLQESGPGLVKPSQTLSTCTVSGGSISSGGYYWNIHQHPGKGLEWIGYIYVSGSTYYNPSLKSRVTISVDTSKNQFSLKLSVTAADTAVYYCARHRAASGSPDADYWGQGTITVTVSS

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